ELECTRON-TRANSFER REACTIONS OF CYTOCHROME b-562 AND ITS MUTATED MOLECULES AT AN INDIUM OXIDE ELECTRODE

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Electron transfer reactions of metalloproteins at functional electrodes are currently interested in bioelectrochemistry and related fields [1]. Myoglobin (Mb) electrochemistry, for example, can be measured at an indium oxide electrode with a highly hydrophilic surface, and its biological functions and properties of reconstituted and modified Mbs have been studied electrochemically [2–7]. Cytochrome b-562 [8] is an interesting electron-transfer heme protein. When the Met-7 of cytochrome b-562 was mutated with Ala (M7A) or Gly (M7G), the heme co-ordination structure is expected to be changed and the coordination site became open for an exogenous ligand to give myoglobin functions.

In the present study, electron transfer reactions of cytochrome b-562 and its mutated molecules were examined by using an In2O3 electrode.

Cytochome b-562 was prepared using *E-coli*, and Met-7 of Cyt b-562 was changed by Ala and Gly using the site-directed mutagenesis technique. To make the structure of mutated molecules stable, both Glu-4 and Glu-8 were also changed at the same time to Ser, ie., E4S/M7A/E8S (SAS) and E4S/M7G/E8S (SGS). Raman spectra of mutated molecules showed that native cytochrome b-562 is hemi-chrome type, while mutated molecules are in an aquo-met form. The UV-visible and Raman spectra were obtained using a Shimadzu UV-2100 spectrophotometer and a JASCO NR-1100 spectrometer, respectively.

Cyclic voltammograms (CV) were obtained at 25 ˚C using a BAS CV-50W electrochemical analyzer at an In2O3 electrode (approximately 5 x 5 mm^2, from Kinoene Optics Corp., Japan) under N_2 atmosphere.

Native cytochrome b-562 (WT) and mutated molecules (SAS and SGS) showed well-defined quasi-reversible redox waves at an In2O3 electrode with the highly hydrophilic surface in a 0.1 M bis-Tris buffer solution (pH 6.5), as in the case of native Mb [2]. The $E^{0'}$ value of WT was –1 mV (vs. Ag/AgCl) and the formal heterogeneous electron transfer rate constant, $k^{0'}$, of 5 x 10^{-3} was obtained from the observed voltammograms by using the digital simulation technique. Importantly, electrochemical method has an advantage to obtain electron transfer kinetics without any variation of the driving force independent of the redox potentials of metalloproteins of interest. The obtained $E^{0'}$ and $k^{0'}$ values were, -115 mV and 1.8 x 10^{-3} cm s^{-1} for SAS, and –120 mV and 1.3 x 10^{-3} cm s^{-1} for SGS, respectively. Taking into account that the heme co-ordination structure, it is reasonable for the mutated molecules that the redox potentials became more negative and the $k^{0'}$ values became smaller compared with those values of WT, because the mutated molecules have an aquo-met form. A water molecule is co-ordinated to the heme iron in their oxidized forms and in their reduced forms water molecule is released from the heme iron, like the case of myoglobin. The H-bond network of water in the heme pocket would affect the electron transfer kinetics [6, 9]. The larger $k^{0'}$ value for WT was observed because WT has Met-7 as the sixth ligand of the heme and no significant reorganization of the heme environment is required during the electron transfer reaction.

Both SAS and SGS accepted an exogenous ligand in their oxidized forms and very similar dissociation kinetics for cyanide to native myoglobin during electron-transfer reaction, meaning mutated cytochrome b-562 molecules have a myoglobin function.

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